QUALITATIVE AND QUANTITATIVE ANALYSIS OF NATURAL COLORANTS FROM BANANA BRACTS

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ABSTRACT

Natural colorants are preferable nowadays due to their beneficial health values. Natural colorants have been used as one of the colorants in food. The banana bracts, scientifically known as *Musa X paradisiaca* will be used in this study as the raw material. This study aimed on determining the best concentration of methanol in methanol-water, the best temperature to maintain the colorants in the samples extracted and the best storage condition. The banana bract layers will be washed and cut into smaller pieces. The colorant contains in banana bracts, known as anthocyanins will be extracted by blending 10g of banana bracts into the solvent until the bracts fully blended in a blender. Then, the sample extracted will be filtered to remove the residue of banana bract layers. Rotary evaporator were used to remove all methanol from the solutions extracted. Every sample was stored for 3, 6, 9, 12 and 15 days in a dark place. The characteristics of colorants were analyzed by using UV-Vis spectrophotometer. Qualitative analysis was based on the ability of the colorants to stands for 15 days. From the analysis, the best concentration of methanol in methanol- water if the sample was stored in the light was 30% at the temperature of 70°C. When the sample was stored in the dark, the best concentration of methanol in methanol-water was 40% at the temperature of 50°C. Qualitatively, all the samples could last up to 15 days.

ABSTRAK

Pewarna semulajadi menjadi pilihan pada hari ini kerana faedah nilai kesihatannya. Pewarna semulajadi telah digunakan sebagai salah satu pewarna di dalam makanan. Jantung pisang, yang secara saintifiknya dikenali sebagai *Musa X Paradisiaca*, akan digunakan sebagai bahan mentah di dalam kajian ini. Kajian ini bertujuan untuk menentukan kepekatan metanol terbaik di dalam metanol-air, suhu terbaik untuk mengekalkan pewarna di dalam sampel yang diekstrak dan keadaan simpanan terbaik. Lapisan jantung pisang akan dikeringkan di bawah cahaya matahari setelah dibersihkan dan dipotong kecil. Pewarna yang terkandung di dalam jantung pisang, dikenali sebagai *anthocyanins*, akan diekstrak dengan cara menghancurkan 10 gram jantung pisang ke dalam pelarut sehingga semua jantung pisang hancur sepenuhnya. Kemudian, sampel yang diekstrak akan ditapis untuk membuang sisa-sisa lapisan jantung pisang. Penyejat berputar telah digunakan untuk membuang semua metanol daripada larutan yang diekstrak. Setiap sampel akan disimpan selama 3, 6, 9, 12 dan 15 hari di dalam tempat yang gelap. Ciri-ciri pewarna dianalisa dengan menggunakan UV-Vis spectrophotometer. Analisis kualitatif adalah berdasarkan kemampuan pewarna untuk tahan selama 15 hari. Berdasarkan analisis, kepekatan metanol yang terbaik di dalam metanol-air jika sampel disimpan di dalam terang adalah 30% pada suhu 70°C. Apabila sampel disimpan di dalam gelap, kepekatan metanol terbaik adalah 40% pada suhu 50°C. Secara kualitatif, semua sampel boleh tahan sehingga 15 hari.

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g	-	gram
ml	-	milliliter
%	-	percentage
°C	-	degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Natural colorants were used as attraction to food products. The demands for natural colorants become higher due to safety issues. During ancient time, those people used saffron for its flavor and colorants. In 19th century, many problems occur as a result from the usage of food colorants that made from minerals. Lead chromate and copper sulphate which were used as food colorants during that time had caused deaths because they were contaminated with arsenic. The use of synthetic colorants is becoming less important as they are unsafe and can cause health problems (Vargas and Lopez, 2003).

Nowadays, color additives are widely used as to (1) restore the original food appearance, (2) ensure color uniformity, (3) intensify colors normally found in food, (4) protect other components (such as antioxidants), (5) obtain the best food appearance, (6) preserve characteristics associated with food, and (7) help as a visual characteristic of food quality (Vargas and Lopez, 2003).

Food colorants may be divided into three types, depending on their source (Vargas and Lopez, 2003):

(a) Natural colors, organic colorants derived from natural edible sources such as anthocyanins;

(b) Nature-identical colors, manufactured by chemical synthesis so as to be identical to colorants found in nature (e.g. β-carotene and riboflavin);

(c) Synthetic colors that do not occur in nature, produced by chemical synthesis (e.g. tartrazine and carmoisine).

In this study, banana bracts (*Musa X paradisiaca*) are used as raw material to extract its natural colorants which is anthocyanins. Most bananas have red, purple or violet bracts although a few are acyanic- green or yellow. The variation in bract color is correlated with the composition of the anthocyanins present, which is distinctive of species and sub species (Duran *et al*., 2000). Banana bracts were usually thrown away as waste during harvesting season back then. Recently, anthocyanin pigments in banana bracts were found as potential food colorants. Anthocyanins have become a good choice for food colorants as it is easily dissolve in water. New sources of anthocyanins with high stability and low cost are desired as natural food colorants (Duran *et al*., 2000).

1.2 Problem Statement

Synthetic dyes were more preferable than natural colorants. There were lots of studies on natural colorants before but they were not used widely. This is because natural colorants are unstable as they are easily influenced by pH value, temperature and presence of oxygen, enzymes and condensation reactions. In this study, the qualitative and quantitative properties of natural colorants will be determined.

The objective of doing this study is to determine and to select which sample can extract the most colorants from the banana bracts and last the longest. Temperature and concentration of methanol in methanol-water will be the parameters

to determine the best sample of colorants so that it can be used for food industry in Malaysia.

1.3 Objectives

The purpose of this study is to determine the best storing temperature of sample and the best concentration of methanol in methanol-water during extraction process in order to see which sample can be stored longer and the color can stands longer. In this study, anthocyanin is the colorant contains in banana bracts.

1.4 Scope of Study

Raw material that is to be used in this experiment is banana bracts. Banana bracts are very easy to get as it comes from banana tree. The colored layers of banana bracts are easily obtained because banana is one of local fruits. The layers of banana bracts are taken and washed. Banana bracts need to be dried so that the moisture is eliminated before processing them.

In the extraction process, anthocyanins were extracted with varied concentration of methanol in methanol-water. Methanol concentrations to be used in this study are 10%, 20%, 30% and 40%. The layers of banana bracts are blended with the solvent in a blender.

After the blending process is done, all samples will be stored in the water bath for 30 minutes at room temperature (30°C), 50°C, 60°C, 70°C and 80°C. Four samples will be stored at room temperature to see the ability of the color to stands for 15 days.

After extraction is completed, every sample will be put in a rotary evaporator to eliminate all the methanol contains in every sample. Then, every sample will be put in the water bath accordingly. UV-Visible spectrophotometer is used to determine the color absorbance of the sample.

1.5 Rationale and Significance of this Study

The rationale of doing this study is banana bracts are easily found. The layers of banana bracts are usually thrown away when people want to cook them. Rather than wasting them, they can be useful as a natural source of natural colorants. This source of anthocyanins is low cost making it desired as natural food colorants.

The significance of doing this study is to investigate the best condition to extract colorants from banana bracts in order to make it last long. If the colorants can last long, it can be used for a longer period. Besides, this study is done to determine the intensity of colorants exist in banana bracts. The optimum anthocyanins in a sample will be chosen to be the best sample and can be used as colorants in food production. The outcomes from this study can also open up opportunities to increase the demand of natural colorants for food industry in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.6 Banana bracts

Banana bracts or well-known as banana hearts are a part of banana tree with purple layers of skin. In Malaysia, the people especially Malays used banana bracts for culinary purposes. Banana bracts are also available at supermarkets as Malaysia is one of the banana's producers. Since the bracts of banana are widely available and have been traditionally used as food without apparent toxic effect, they could be a potential source of anthocyanins (Duran *et al.*, 2000) to be natural colorants.

Figure 2.1: Banana bracts (laocook.com)

Most bananas have red, purple or violet bracts although a few are acyanic-green or yellow. The variation in bract color is correlated with the composition of the anthocyanins present, which is distinctive of species and subspecies (Durán *et al.*, 2000).

2.2 Anthocyanins

Anthocyanins are natural colorants which have raised a growing interest due to their extensive range of colours, innocuous and beneficial health effects. Despite the great potential of application that anthocyanins represent for food, pharmaceutical and cosmetic industries, their use has been limited because of their relative instability and low extraction percentages. Currently, most investigations on anthocyanins are focused on solving these problems, as well as their purification and identification (Ovando *et al*., 2008). The isolated anthocyanins are highly instable and very susceptible to degradation (Giusti & Wrostlad, 2003). Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins and metallic ions (Rein, 2005).

Anthocyanins are flavonoid phenolic compounds, widely distributed among fruits, berries and flowers, providing attractive colours, such as orange, red and blue. These pigments are water-soluble and this property facilitates their incorporation into numerous aqueous food systems. These qualities make anthocyanins attractive natural colorants. Moreover, it has been demonstrated that, in addition to their colourful characteristics, anthocyanins possess some positive therapeutic effects, mainly associated with their antioxidant properties (Longo and Vasapollo, 2004). Anthocyanins have recently received increasing attention as natural colorants in food systems, as a consequence of the social trend toward the consumption of natural products instead of synthetic ones. Thus, new sources of pigments, such as anthocyanins, with high colorant power, stability and low cost are nowadays desired (Longo and Vasapollo, 2004).

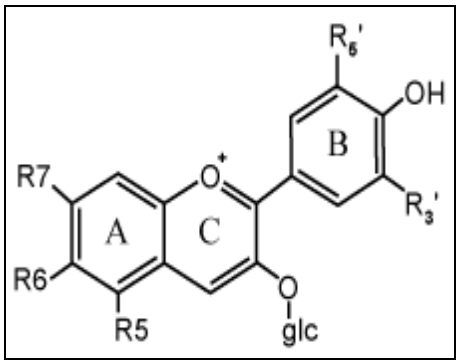

Figure 2.2: General anthocyanin structure (www.micro-ox.com)

The anthocyanin structures produce a great range of colors from scarlet to blue that are clearly represented in flowers and fruits, although they are also present in leaves and storage organs. Anthocyanins are common in higher plants but are absent in some lower plants such as liverworts and algae (Vargas and López, 2003).

According to the numbering system used by the Codex Alimentarius Commission, anthocyanins (any anthocyanin-derived colorant) are listed as a natural colorant by the European Union (EU) legislation as product E163. With respect to the US, the FDA (Food and Drug Administration) has a different list of "natural" colors that do not require certification (without any FD & C numbers), and anthocyanins can be obtained either from "grape color extract", "grape skin extract", "fruit juices or vegetable juices". Nevertheless, the use of anthocyanins in food products is restricted to some products varying among countries. Usually, the USA is the most restrictive country regarding the use of anthocyanin colorants (Gould *et al*., 2009).

Anthocyanins are interesting pigments regarding their chromatic features. The oldest anthocyanin extract used in the food industry is enocyanin obtained from red grape pomace and marketed in Italy since 1879. Nowadays, grape extracts are often used as colorants for sugar confectionary, dairy products, ice creams, etc (Gould *et al*., 2009).

2.3 Natural colorants

Food coloring (colouring) is any substance that is added to food or drink to change its color. Food coloring is used both in commercial food production and in domestic cooking (www.wikipedia.org). Natural colorants are colors that can be obtained from natural sources such as fruits and vegetables. Colors that occur in these natural sources are caused by the content in them.

Natural colorants were not an option for food colorants back then because of its stability. They are water-soluble, which facilitates their incorporation into aqueous food systems. They are widespread in nature and are innocuous. These qualities make them attractive natural colorants. However, some factors, such as the low stability when compared with synthetic dyes have limited their use (Durán *et al.*, 2001).

Colour is an important factor in the acceptability of a food product. The safety of synthetic colorants has previously been questioned, leading to a reduction in the number of permitted colorants. Due to this limitation and to the worldwide tendency towards the consumption of natural products, the interest in natural colorants has increased significantly (Durán *et al.*, 2001).

2.4 Rotary Evaporator

The rotary evaporator is a device for gently and efficiently evaporating solvents from a mixture. It consists of a heated rotating vessel (usually a large flask) which is maintained under a vacuum though a tube connecting it to a condenser. The rotating flask is heated by partial emersion in a hot water bath. The flask's rotation provides improved heat transfer to the contained liquid; the rotation also strongly reduces the occupance of 'bumps' caused by superheating of the liquid. The solvent vapors leave the flask by the connecting tube and are condensed in the condenser section. The condenser section is arranged so that the condensed vapors drain into

another flask where they are collected. It is a very efficient way of rapidly removing large quantities of solvent. The major use in chromatography is the recovery of non-volatile solutes in preparative chromatography and the recovery of solvents for recycling. The device is also used for preparing coated supports for gas chromatography. For this application a weighed amount of support is placed in the flask, the required amount of stationary phase dissolved in excess of solvent is added to the solid and mixture tumble dried. This procedure has two advantages; as well as drying the support it ensures a very even distribution of the stationary phase throughout the support (chromatography-online.org).

A vacuum is a key component of this device, and is used to aid in the evaporation of the solvent. A vacuum will lower the air pressure in the space above a liquid, thereby lowering the boiling point of the liquid being heated. Often the mixture is very sensitive and highly reactive so excessive heating would not be ideal. The vacuum allows the user to temporarily alter the physical properties of the sample so that solvents can be extracted from a variety of mixtures that would otherwise be impossible. The simplest type of vacuum used on a rotary evaporator is a water aspirator. Picture a tube that is wider at the top and narrows as it goes down, with an opening at the side. When water is channeled downwards, it increases speed, and the pressure is lowered - creating a vacuum effect through the opening at the side. A more complex method would involve a regulated mechanical vacuum pump (article-niche.com).

Rotation is not absolutely necessary for the evaporation process to take place. However, the centrifugal forces allow the solvent to rise and be spread thinly over a larger surface, which makes it easier to extract. Also, the rotation helps reduce “bumping.” Bumping is what happens when two substances come together, like water and ethanol, and the total amount is reduced. This is due the difference in the size of the molecules (article-niche.com).

The rotary evaporator has evolved over the years to become more and more advanced, and while features and capabilities of different models vary widely, modern rotary evaporators still consist of seven primary components (article-niche.com):

• The motor that rotates the sample (contained in either a flask or vial)
• Hot water bath to heat the sample
• The vacuum used to lower the pressure
• A vapor duct to capture the solvent
• Condenser that cools the solvent
• Flask to collect the solvent after it condenses
• A mechanism to quickly remove the sample from the hot water bath

2.5 Color Measurement

The principal attributes of object colors are hue, lightness, and saturation (Vargas and López, 2003). Hue is the quality that we normally identify with a color name such as red, green, and blue. Lightness is a term related to the concept of light and dark by considering color as a source of reflected light. Lightness is the light reflected by a surface in comparison to a white surface, under similar conditions of illumination. A related term is brightness, but this is used for the total light from the illuminant or reflected from a surface. Lightness and brightness are grouped in the term value, although lightness and value are commonly used interchangeably. Saturation is the clarity or purity of a color. Also, it can be understood as the intensity of hue in comparison to its own brightness. A saturated color looks clear and bright, but an unsaturated color appears pale, muddy, or dull (Vargas and López, 2003).

2.5.1 UV-Visible Spectrophotometer

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, b, and the concentration, c, of the absorbing species. Beer's Law states that

A = **ebc**, where **e** is a constant of proportionality, called the absorbtivity (teaching.shu.ac.uk).

Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. For example, the absorption that is observed in the UV region for the carbonyl group in acetone is of the same wavelength as the absorption from the carbonyl group in diethyl ketone (teaching.shu.ac.uk).

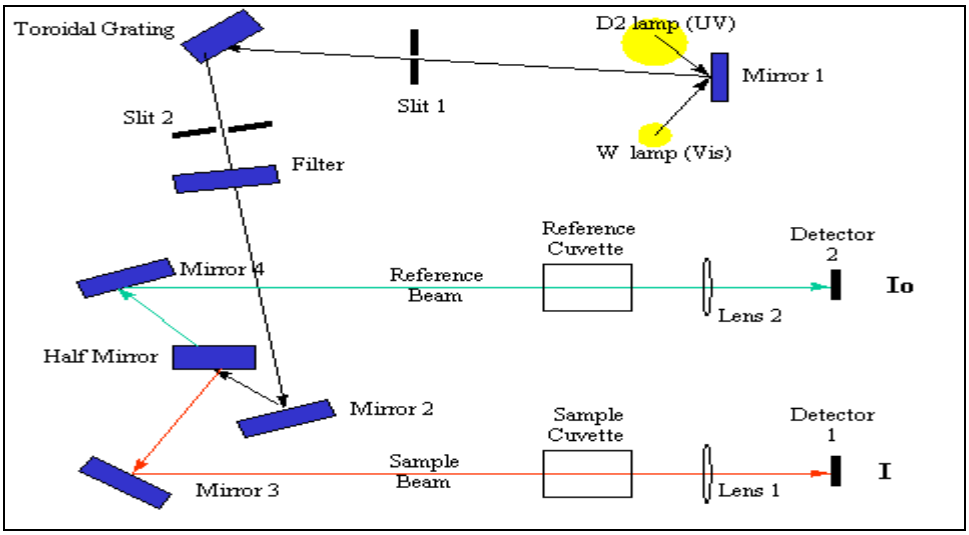

Figure 2.3: Optical System Diagram for UV-Visible spectrophotometer

The UV-Visible spectrophotometer uses two light sources, a deuterium (D_2) lamp for ultraviolet light and a tungsten (W) lamp for visible light. After bouncing off a mirror (mirror 1), the light beam passes through a slit and hits a diffraction grating. The grating can be rotated allowing for a specific wavelength to be selected. At any specific orientation of the grating, only monochromatic (single wavelength) successfully passes through a slit. A filter is used to remove unwanted higher orders

of diffraction. (Recall the experiment you did last semester on Atomic Spectra) The light beam hits a second mirror before it gets split by a half mirror (half of the light is reflected, the other half passes through). One of the beams is allowed to pass through a reference cuvette (which contains the solvent only), the other passes through the sample cuvette. The intensities of the light beams are then measured at the end.

CHAPTER 3

METHODOLOGY

3.1 Materials and Solvents

The banana bract species in this study is named *Musa X paradisiaca* from Malaysia that bought from the night market in Gambang. The solvent used to extract the colorants is methanol and water. Methanol and water will be mixed and used to extract the colorants from banana bracts.

3.2 Apparatus

For drying process, banana bracts layers will be washed and dried to remove the moisture content in the banana bracts. Besides, it is easier to store a dry banana bract to prevent it from being damaged. In the extraction process, methanol-water is used to pull out the colorants from the banana bracts. A blender was used to blend the layer of banana bracts. Absorbance of the colorants will be determined by using UV- Visible Spectrophotometer. Another equipment to be used is rotary evaporator. This equipment is needed to remove the content of methanol in all samples of extracted banana bract colorants.

3.3 Sample Preparation

The colored layers of banana bracts are to be taken. They need to be washed with cold water to remove any dirt on the layers. The layers will be cut into smaller pieces before the blending process. Then, wipe the banana bracts to remove all the water. The banana bracts can now be used for extraction procedure.

Figure 3.1: Sample preparation

3.4 Extraction

After the solvents are prepared, extraction process can be done. 10 g of layers of banana bracts will be put in the solvents and will be blended in a blender. The concentration for every sample will be different; 10%, 20%, 30% and 40% methanol in methanol-water.

Figure 3.2: Extracted samples

3.5 Storage and analysis

When the extraction process was finished, the solvent will be removed by using Büchi rotary evaporator. By using rotary evaporator, methanol was eliminated. The temperature used for the elimination of methanol is 70°C, which is higher than the boiling point of methanol. After all methanols were eliminated, the samples were stored.

Figure 3.3: Rotary evaporator

After the elimination of methanol, the samples will be put in a water bath at different temperature. The temperature used was varied ; 50°C, 60°C, 70°C and 80°C. All samples were stored in the light and in the dark.

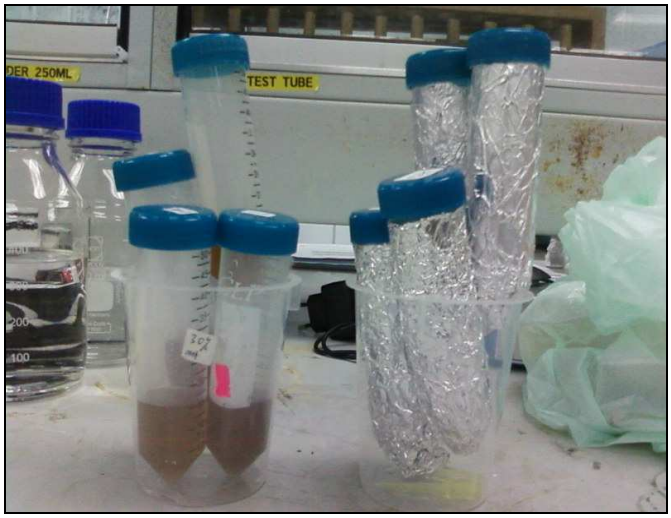

Figure 3.4: Samples to be stored

After every 30 minutes, samples that were stored in the light and in the dark were analyzed by using UV-Vis spectrophotometer. UV-Vis spectrophotometer analyzed the samples and showed the value of absorbance for each of the samples. The wavelength used was 700 nm. From the value of absorbance, concentration of colorants in the samples was determined.

Figure 3.5: UV- Visible Spectrophotometer